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Full Length Article

Seed Priming Modulates Germination Potential, Osmoprotectants Accumulation and Ionic Uptake in Wheat Seedlings under Salt Stress

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Abstract

Increasing salinity is a major threat to crop growth and development while seed priming can effectively induce salt tolerance in plants. The present study examined the role of ascorbate priming (ascorbate; 50 mg L^{-1}), hormonal priming (triacontanol and indole acetic acid; 25 and 100 μmol, respectively) and osmolyte priming (proline; 50 m*M*) in wheat seedling grown under NaCl based salinity (12 dS m^{-1}) as well as normal (3 dS m^{-1}) conditions. The results indicate that priming with triacontanol (TRIA) followed by ascorbate (AsA) priming alleviated the detrimental effects of salinity stress by reducing germination time accompanied by improved germination index and final germination. Enhanced seedling growth in terms of better root and shoot lengths, seedling fresh and dry biomass were recorded in primed seeds. Under both conditions, all priming treatments successfully improved physio-biochemical traits. Maximum chlorophyll, glycine betaine and potassium contents were recorded in seedlings obtained from seeds primed with AsA and TRIA priming. Pre-soaking with IAA produced highest proline and total soluble sugars while osmolyte priming showed minimum Na^+ contents and maximum K^+/Na^+ ratio as well as total phenolic contents under salt stress. Plant biomass was positively linked with chlorophyll stability and osmoprotectants accumulation under saline conditions. In crux, triacontanol and ascorbate are the most effective priming agents to induced salt tolerance in wheat, attributed to improved germination with vigorous stand and decreased salt damage due to enhanced osmoprotectants.

Keywords: Seedling establishment; Wheat; Chlorophyll; Compatible solutes; Ascorbate; Hormonal priming

Introduction

Salinity stress imposes a key environmental threat to agriculture. About 7% of the world lands are saline and 3% are highly saline, even so growing because of low precipitation, high evaporation and irrigation by saline waters (Gupta and Huang, 2014). Salinity being one of the most serious environmental stress factor affecting wheat *(Triticum aestivum* L.) cultivation in arid and semi-arid regions including Pakistan (Mahboob *et al*., 2016), where grain yield losses from salt-affected lands ranged from 20–43% with an overall average loss of 32% (Murtaza, 2013). Salt stress affects almost all stages of wheat growth and development however seed germination and synchronized stand establishment are the most sensitive developmental phase to salinity (Kochak-Zadeh *et al*., 2013).

Salinity decreases the potential of plants to take up water and rapidly results in reduced growth rate, along with a series of metabolic changes like those caused by drought stress (Abbasdokht, 2011; Farooq *et al*., 2015; Mahboob *et al*., 2018). Numerous physiological and biochemical changes occur in response to salt stress including restricted water uptake (Park *et al*., 2016; Farooq *et al*., 2017), degradation of chlorophyll and ionic imbalance (Mahboob *et al*., 2016), reduction in enzyme activities (Yucel and Heybet, 2016), disturbance in the cellular redox homeostasis which leads to enhanced production of reactive oxygen species (Das and Roychoudhury, 2014). Salinity stress alters the patterns of Na^+ and K^+ accumulation; thus, greater K^+/Na^+ ratio is more significant for numerous plant species than simply maintaining a low concentration of Na^+ (Munns and Tester, 2008; Farooq *et al*., 2015, 2017; Shirazi *et al*., 2018).

There are many strategies to overcome the negative impacts of salt stress on plant growth and yield. Seed priming is a safe, low cost and highly effective way to mitigate saline stress in plants particularly during germination and plant growth at early stages (Afzal *et al*., 2006; Jafar *et al*., 2012; Afzal *et al.*, 2013). Seed priming alters germination metabolism by reducing time period from planting to emergence to shelter the seeds from abiotic factors during important stage of seedling establishment, so as to synchronize emergence, which results in uniform stand and better yield (Ashraf and Foolad, 2005; Afzal *et al.*, 2011). Many seed priming strategies have been employed to enhance stand establishment, seedling growth and to improve physio-biochemical characteristics in wheat grown under optimal as well as saline areas (Afzal *et al*., 2006;

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Jafar *et al*., 2012; Yucel and Heybet, 2016). Seed priming stimulates the accumulation of osmoprotectants by modulating metabolic processes and lowers the Na^+ contents (Gupta and Huang, 2014; Tabassum *et al*., 2017, 2018; Bajwa *et al*., 2018; Naqve *et al.*, 2018).

Numerous studies demonstrated that potential of seed germination in different plant species can significantly be improved via plant growth regulators or other organic substances under optimal as well as stressed conditions (Ashraf and Foolad, 2005). It is well documented that auxins have ability to alleviate the inhibitory effects of salinity, thus can be used to improve wheat seed germination and plant growth (Iqbal and Ashraf, 2007). Improved seedling emergence, enhanced root and shoot length, fresh and dry weights and significant amelioration in physio-biochemical attributes in wheat were resulted from exogenous application of ascorbate (Afzal *et al*., 2006; Jafar *et al*., 2012) proline (Ashraf and Foolad, 2007; Kamran *et al*., 2009; Mahboob *et al*., 2016) and triacontanol (Cavusoglu *et al*., 2007; Perveen *et al*., 2012) under optimal and saline conditions. Although, salt tolerance induced by seed priming has already been documented in some crops, however information regarding physiological and biochemical basis of priming-induced beneficial effects in wheat under salt stress need to be explore. Therefore, the aim of present investigation was to appraise the efficacy of priming agents on wheat stand establishment and seedlings growth on the basis of osmolytes accumulation through altered metabolic processes when subjected to salt stress.

Materials and Methods

Experimental Details

The study was conducted at Plant Physiology Laboratory, Nuclear Institute of Agriculture (NIA), Tandojam, Pakistan. Seeds were placed on moulded nets floating in hydroponic nutrient solution in plastic pot (width 8.7 cm x height 4.8 cm) under controlled conditions in growth chamber (Vindon; 8194, England). Day and night lengths at 14/10 h, with 25°C and 20°C temperatures, respectively with 60% relative humidity were maintained during experimentation. Layout of the experiment was complete randomized design (CRD), with factorial arrangement of five priming treatments under two salinity levels, replicated thrice. Salt stress of 12 dS m^{-1} was imposed by using NaCl through full strength Hoagland solution while 3 dS m^{-1} nutrient solution was used as control treatment. Twenty seeds were sown in each plastic pot and after ten days of germination, samples were collected to record data for different attributes.

Seed Priming Protocol

Uniform sized healthy seeds cv. Khirman were selected and sterilized in 3% (v/v) sodium hypochlorite solution for 10 min, thoroughly washed by distilled water and air-dried at room temperature. These sterilized seeds were then soaked in aerated solutions of ascorbate (50 mg L^{-1}; ascorbate priming), proline (50 m*M*; osmolyte priming), triacontanol and indole acetic acid (25 and 100 μmol, respectively; hormonal priming) for 12 h by maintaining 1:5 (w/v) seed to solution ratio, while untreated seeds were used as control (NP). After priming, seeds were then air dried up to the level of initial moisture content (~12%). Untreated seeds were used as control (NP).

Measurements

Germination and seedling growth: Seed germination was noted on daily basis up till the final germination count was retained. Germination metabolism related traits including time required for 50% germination (G_{50}), mean germination time (MGT), germination index (GI) and final germination percentage (FGP) were computed from the germination data following the standard equations of Coolbear *et al*. (1984), Ellis and Roberts (1981) and handbook of AOSA (1990), respectively.

Five plants were selected from each treatment to record growth parameters. Shoot and root lengths were assessed with the help of measuring rod. Electrical weighing balance (AND-3000; Japan) was used for recording the seedling fresh weight (fig. 2c), while dry weight was obtained after drying in an oven (Sanyo; MOV-202F) at 70°C for 72 h.

Estimation of biochemical attributes: Leaf chlorophyll contents were measured according to the method proposed by Arnon (1949). Compatible solutes i.e. Free proline, glycine betaine, total soluble sugars and leaf phenolics were measured in next to flag leaf on fresh weight basis by following the methods proposed by Bates *et al*. (1973), Grieve and Gartan (1983), Riazi *et al*. (1985) and Waterhouse (2001), respectively.

Determinations of ionic contents: For ionic (Na^+ and K^+) analysis, grinded dry leaf samples (0.1 g) were extracted in acetic acid (0.1 *M*) for one hour in water bath pre-heated at 95°C. Sodium and potassium concentrations were determined in suitable dilution by using flame photometer (Jenway; PFP-7, England).

Statistical Analysis

Fisher analysis of variance technique was employed to analyze experimental data and significant treatments means were examined by least significance difference (L.S.D.) test at 0.05 probability levels (Steel *et al*., 1997). Graphical representation of stand establishment and seedling growth data were made by using Microsoft Excel program (Microsoft Corporation, Los Angeles, CA, USA) while IBM-SPSS Statistics (V21 x86) was used to draw the correlation among the treatments under salinity.

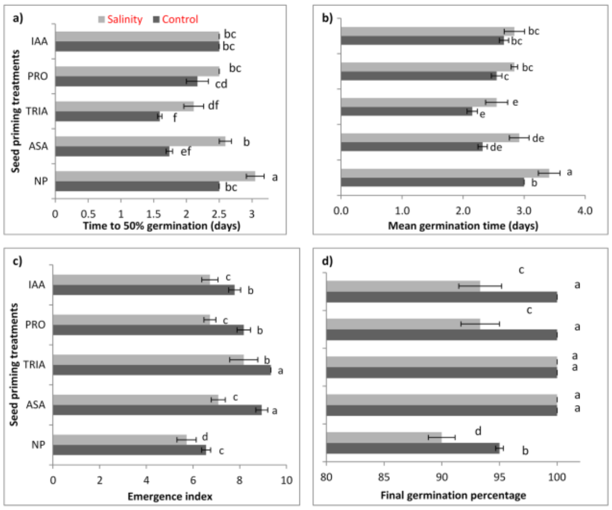

Fig. 1: Effect of priming treatments on stand establishment traits of wheat seedlings under salt stress. Bars sharing the same letter, for a parameter, do not differ significantly at $P \leq 0.05$. NP; Non-primed: ASA; ascorbic acid: TRIA; triacontanol: Pro; proline, IAA; indole acetic acid

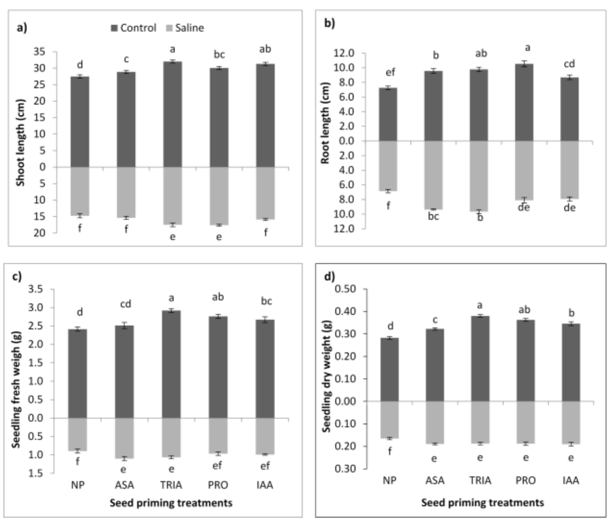

Fig. 2: Impact of seed priming treatments on agronomic traits of wheat seedlings under salt stress. Bars sharing the same letter, for a parameter, do not differ significantly at $P \leq 0.05$. NP; Non-primed: ASA; ascorbic acid: TRIA; triacontanol; Pro: proline; IAA: indole acetic acid

Results

Germination and Stand Establishment

Salinity stress had exerted adverse effects on germination, root and shoot growth and physiological responses of wheat seedlings. Higher concentration of NaCl in water culture medium had reduced the rate of germination. However, priming treatments significantly enhanced germination processes in terms of reduced time taken to G_{50}, mean germination time (MGT) and improved germination index (GI) as well as final germination (Fig. 1). Among the soaking treatments, hormonal priming with triacontanol (TRIA) took least time to complete G_{50} as well as minimum MGT over control and other treatments under all experimental conditions. Under salinity non-primed seeds took maximum time to germinate by showing higher values for G_{50} and MGT (Fig. 1a, b). Conversely, all the priming treatments had significantly improved germination index and final germination as compared to control under optimal and saline conditions (Fig. 1c, d). Maximum germination index and final germination were noted for seeds exposed to TRIA and ascorbate priming under normal as well as stressed conditions.

Salt medium caused a reduction in the root and shoot lengths, seedling fresh and dry weights. Seed priming significantly improved early seedling growth by mitigating the adverse effects of salinity (Fig. 2). Priming with TRIA and Pro illustrated maximum root and shoot length, respectively, as compared to non-primed seeds under salt stress. Ascorbate and IAA were unable to enhanced shoot growth and behaved alike control while all priming treatments produced significant root length under saline environment (Fig. 2a, b). However, TRIA priming was most effective in improving seedling fresh and dry weight in optimal conditions. Under saline medium, seedlings rose from ascorbate and triacontanol priming revealed maximum fresh weight while all priming treatments had significantly improved seedling dry weight over untreated control under salt stress as well as optimal conditions (Fig. 2d).

Biochemical Attributes

A significant degradation in chlorophyll pigment was observed under salt stress (Table 1). Seedlings obtained from priming treatments illustrated better leaf chlorophyll contents as compared to non-primed under all experimental conditions. However, ascorbate priming was most effective in maintaining highest chlorophyll *a* content in normal and saline medium. On other hand, maximum chlorophyll *b* was recorded in response to Pro and IAA priming in non-saline condition, while TRIA priming reduced salinity-induced degradation of chlorophyll and gave maximum value for chlorophyll *b* related to other treatments.

The endogenous concentrations of different osmoprotectants *i.e.*, Pro, GB, TSS and total phenolics positively influenced by pre-sowing treatments as well as salt stress but at varying degree (Table 1). All priming treatments increased proline content; however highest free proline content was produced by IAA under normal as well as stressed conditions. Osmolyte priming failed to accumulate significant proline content over control under non-saline medium but showed significant improvement by 8.6-folds more proline under salt stress. Like proline, maximum GB was observed in seedlings obtained from IAA priming in control but surprisingly failed to maintain it under salinity and behaved alike non-primed, hence the utmost accumulation of GB was resulted from ascorbate priming followed by TIRA under salt stress (Table 1). Seedlings obtained after priming showed differential behavior regarding TSS accumulation when exposed to salt stress. Priming with IAA and TRIA produced highest TSS respectively, while ascorbate and osmolyte priming had not illustrated significant difference over NP control. Among all treatments, Pro priming proved most effective and exhibited highest total phenolic contents under all experimental conditions (Table 1).

Data for leaf ionic content showed that priming

Table 1: Impact of various priming techniques on chlorophyll stability and osmoprotectants accumulation in wheat seedlings under salt stress

Priming Treatments	Chlorophyll *a* (mg g^{-1})		Chlorophyll *b* (mg g^{-1})		Proline (μmol g^{-1})		Glycine betaine (μmol g^{-1})		Total soluble sugars (mg g^{-1})	
	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity
Non-primed (NP)	0.790 f	0.766 f	0.626 e	0.466 f	4.963 g	38.21 d	55.03 f	55.41ef	3.57 f	11.5 de
Ascorbate (AsA) priming	1.22 a	1.05 cd	0.691 d	0.77 bc	6.190 f	40.30 c	58.72 d	72.49 a	14.3 b	12.2 cd
Hormonal (TRIA) priming	1.14 b	1.00 d	0.722 cd	0.863 a	5.24 fg	41.65 b	58.5 de	65.41 b	11.2 e	14.1 b
Osmolyte (Pro) priming	1.07 bc	1.04 cd	0.833 a	0.67 de	4.89 g	42.37 ab	60.14 cd	62.39 bc	12.7 c	12.2 cd
Hormonal (IAA) priming	1.01 cd	0.901 e	0.824 ab	0.685 d	7.39 e	42.83 a	62.84 bc	55.48 ef	11.4 de	15.8 a
LSD ($P \leq 0.05$)	0.0707		0.0531		0.975		3.229		0.961	

Means sharing the same letter for a parameter do not differ significantly $P \leq 0.05$ by least significant difference test
[a] Non-primed control

Table 2: Impact of various priming techniques on total phenolics and ionic contents of wheat seedlings under salt stress

Treatments	Total phenolics (mg g-[1])		Sodium concentration (%)		Potassium concentration (%)		Sodium/Potassium ratio	
	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity
Non-primed (NP)	2.45 f	3.03 de	2.03 e	2.93 a	2.15 b	1.11 e	1.06 d	0.38 g
Ascorbate (AsA) priming	2.95 e	3.75 bc	1.85 g	2.15 d	1.99 b	1.09 e	1.61 a	0.51 fg
Hormonal (TRIA) priming	2.29 f	4.00 ab	1.63 h	2.67 b	2.63 a	1.74 c	1.07 d	0.65 e
Osmolyte (Pro) priming	2.95 e	4.22 a	1.66 h	1.93 fg	2.05 b	1.28 de	1.23 c	0.66 e
Hormonal (IAA) priming	2.48 f	3.41cd	1.95 ef	2.36 c	2.76 a	1.35 d	1.41 b	0.57 ef
LSD ($P \leq 0.05$)	0.3859		0.097		0.225		0.135	

Means sharing the same letter for a parameter do not differ significantly $P \leq 0.05$ by least significant difference test
[a] Non-primed control

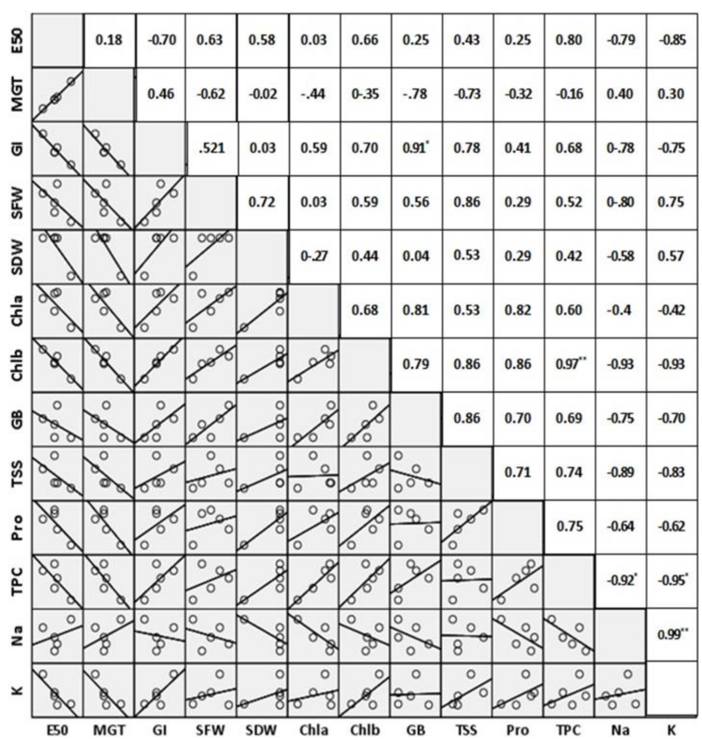

Fig. 3: Correlation coefficients of various morphological, physiological and biochemical traits of wheat under salt stress (n=5)
**= Significant at $P \leq 0.01$; *= Significant at $P \leq 0.05$; $G_{50=}$ Time to 50% germination; MGT= Mean germination time; GI= Germination index; SWF= Seedling fresh weight; SDW= Seedling dry weight; *Chl a*= Chlorophyll *a*; *Chl b*= Chlorophyll *b*; GB= Glycine betaine; TSS= Total soluble sugars; Pro= Proline; TPC= Total phenolics content

treatments had significantly restricted Na^+ uptake as compared to non-primed under all experimental conditions (Table 2). Priming with Pro followed by AsA showed least accumulation of Na^+ under both growing mediums. Except IAA and TRIA, all priming agents failed to improve leaf K^+ content under control and stressed environment. On exposure to saline medium, TRIA and Pro priming showed highest Na/K ratio over non-primed control.

Under saline conditions, seedling fresh and dry weights showed a positive correlation with germination index (GI), photosynthetic pigments, K^+, total phenolics and osmoprotectants, while negatively correlated with stand establishment (G_{50} and MGT) and Na^+ (Fig. 3). In case of stand establishment, GI revealed positive relationship with photosynthetic pigments, K^+, total phenols and osmoprotectants while G_{50} and MGT exhibited a negative correlation with these parameters.

Discussion

Slow seed germination and erratic stand establishment are the foremost effects of salt stress which may result in very low agricultural productivity by adversely affecting plant growth and development (Jafar *et al*., 2012; Mahboob *et al*., 2018). However, seed priming helps to maintain better seedling growth under salinity by repairing seed damages and vigorous germination (Ehsanfar *et al*., 2006). Seed priming significantly improved salt tolerance through better emergence and stand establishment (Fig. 1), which indicates that seed priming triggers hydrolytic enzymes and alters embryo physiology so that germination metabolism can occur more rapidly than normal. Charlton *et al*. (1980) disagreed with present findings that TRIA significantly improved germination in wheat (Fig. 1), however confirmed by those results reported by Cavusoglu *et al*. (2007) where pre-soaking with TRIA has alleviated the adverse effects of salt stress on germination and fresh biomass of barely. Healthy seedling stands obtained after priming might be due to activation of multiple enzymes causing hydrolysis to break seed dormancy, which are the fundamentals of germination (Aziza *et al*., 2004).

Salt stress caused a significant reduction in seedlings growth. Seed priming agents improved plant growth in terms of enhanced root and shoot lengths, seedlings fresh and dry biomass (Fig. 2) which is attributed to their role in maintaining high levels of auxin and cytokinin in plants, that causes cell multiplication (Sakhabutdinova *et al*., 2003). Maximum seedling growth resulted from TRIA (Fig. 2; Perveen *et al*., 2012), could be due to its role to stimulate 9-ß-l (+)-adenosine production, which regulates multiple physiological processes, resulting in enhanced plant growth (Ries *et al*., 1993). Moreover, it has potential to enhance plant metabolism and growth processes by influencing the enzymes involved in carbohydrate metabolism (Singh *et al*., 2011). Better seedling growth from TRIA primed seed was contributed due to rise of photosynthetic activities as TRIA reduced chlorophyll degradation (Borowski and Blamowsk, 2009). It is well documented that AsA and Pro priming enhanced plant growth in wheat (Athar *et al*., 2009) and barely (Agami, 2014) which confirmed our findings that these priming agents played a vital role in inducing salt tolerance through better osmotic adjustment in wheat.

Accumulation of compatible solutes is an important tolerance mechanism in plants under salt stress (Mahboob *et al*., 2017). Seeds subjected to IAA and osmolyte priming resulted in improved accumulation of free proline and glycine betaine respectively, it could be because of augmented proteolysis or reduced protein synthesis (Mahboob *et al*., 2016). These osmoprotectants play a vital role in osmotic adjustment, stabilizing the structure of organelles and macromolecules in wheat and other field crops (Ashraf and Foolad, 2007; Shahbaz *et al*., 2013; Gupta and Huang, 2014) and showed a positive correlation with plant biomasses (Fig. 3). Likewise, accumulation of sugars is commonly experienced in response to salt stress and seed priming which established their role as an osmoprotectant that stabilizes cellular membrane, carbon storage and scavenging of reactive oxygen species (Gupta and Huang, 2014). Furthermore, sugars might contribute to salt stress tolerance either by serving as osmoticum or as respiratory substrates (Mahboob *et al*., 2017). An increase in total phenolics results from priming treatments establishes their role as regulatory chemicals to trigger the production of various secondary metabolites which control many physiological processes (Jaybhay *et al*., 2010). The accumulation of phenols could be a cellular adaptive mechanism for scavenging oxygen free radicals during stress (Mohamed and Aly, 2008) and also illustrated the induction of secondary metabolism as one of the defense mechanisms adapted by the plants to face salinity.

Salt stress caused a significant increase in leaf Na^+ content accompanied by corresponding decline in K^+ concentration (Mahboob *et al*., 2018; Shirazi *et al*., 2018) but differed significantly in response to seed priming (Jafar *et al*., 2012). Taie *et al*. (2013) also reported comparable reduction in Na^+ with improved K^+ in faba bean obtained after seeds priming with proline. Present investigation indicates that priming with Pro and TRIA were most effective to reduce Na^+ uptake and improved K^+ leaf content under saline medium (Table 2). Decrease in leaf Na content may be attributed to the role of priming agents who modulates net buildup of Na in plant cells by regulating equilibrium between influx via ion channels and efflux through a probable Na^+/H^+ antiporter. Highest K content resulted from TRIA priming in present study endorsed by Shahbaz *et al.* (2013).

Conclusion

From the present study, it can be concluded that all the priming treatments alleviated the inhibitory effect of salt stress on seedling growth of wheat. Among treatments, TRIA and ascorbate priming proved to be most effective in improving salt tolerance in wheat due to better seedling vigor, high accumulation of osmoprotectants as well as increased K^+ and K^+/Na^+ ratio along with decreased accumulation of Na^+ in wheat seedlings.

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